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L18: Entry 1 of 2 File: USPT Aug 1, 2000

DOCUMENT-IDENTIFIER: US 6096313 A
TITLE: Compositions containing immunogenic molecules and granulocyte-macrophage colony stimulating factor, as an adjuvant

Abstract Text (1):
Granulocyte-macrophage colony stimulating factor ("GM-CSF") has been found to act as an adjuvant when administered to subjects. Compositions of GM-CSF and immunogenic compositions are presented, as is the use of GM-CSF alone and in these compositions.

Brief Summary Text (2):
This invention relates to compositions useful in the generation of immune responses especially the classes of molecules referred to as tumor rejection antigen precursors ("TRAPs") and tumor rejection antigens ("TRAs"). The immune response includes, inter alia, the production of antibodies against the TRAPs and TRAs, as well as T cells specific for complexes of TRA and major histocompatibility molecules ("MHCs"). Such T cells and antibodies may be generated, e.g., in a mouse, rat, rabbit, sheep, goat or other non-human animal, and then used in diagnostic methods to identify tumor presence. The compositions may also be used, therapeutically, via administration to a subject afflicted with a cancerous condition or one where cell transformation has taken place, such as melanoma or dysplastic nevi, to provoke an immune response against tumors, cancer cells, and transformed cells.

Brief Summary Text (5):
Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

Brief Summary Text (8):
It appears that tum.sup.- variants fail to form progressive tumors because they elicit an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum.sup.- " variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl, Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum.sup.- cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory which permits them to resist subsequent challenge to the same tum.sup.- variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra). Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983).

Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearson et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

Brief Summary Text (9):
A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytotoxic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and the cells presenting the antigen are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including major histocompatibility antigens, the male specific H-Y antigens, and a class of antigens, referred to as "tum.sup.- " antigens, and discussed herein.

Brief Summary Text (10):
A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum.sup.- variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens--and this is a key distinction--the tum.sup.- antigens are only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum.sup.+, such as the line referred to as "P1", and can be provoked to produce tum.sup.- variants. Since the tum.sup.- phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum.sup.- cell lines as compared to their tum.sup.+ parental lines, and this difference can be exploited to locate the gene of interest in tum.sup.- cells. As a result, it was found that genes of turn variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303

Brief Summary Text (12):
Prior patent application PCT/US92/04354, and U.S. Pat. No. 5,342,774, both of which are incorporated by reference describe inventions involving, inter alia, genes and other nucleic acid molecules which code for various TRAPs, which are in turn processed to tumor rejection antigen, or "TRAs". SEQ ID NOS: 1-26 which are a part of the subject PCT application, present sequences of genes coding for various TRAPs, and the TRA referred to hereafter as MZ2-E, which is derived from MAGE-1 TRAP (SEQ ID NO: 26).

Brief Summary Text (13):
The genes are useful as a source for the isolated and purified tumor rejection antigen precursor and the TRA themselves, either of which can be used as an agent for treating the cancer for which the antigen is a "marker", as well as in various diagnostic and surveillance approaches to oncology, discussed infra. It is known, for example, that tum.sup.- cells can be used to generate CTLs which lyse cells presenting different tum.sup.- antigens as well as tum.sup.+ cells. See, e.g., Maryanski et al., Eur. J. Immunol 12: 401 (1982); and Van den Eynde et al., Modern Trends in Leukemia IX (June 1990), the disclosures of which are incorporated by reference. The tumor rejection antigen precursor may be expressed in cells transfected by the gene, and then used to generate an immune response against a tumor of interest.

Brief Summary Text (14):
In the parallel case of human neoplasms, it has been observed that autologous mixed lymphocyte-tumor cell cultures ("MLTC" hereafter) frequently generate responder lymphocytes which lyse autologous tumor cells and do not lyse natural killer targets, autologous EBV-transformed B cells, or autologous fibroblasts (see Anichini et al.,

Immunol. Today 8: 385-389 (1987)). This response has been particularly well studied for melanomas, and MLTC have been carried out either with peripheral blood cells or with tumor infiltrating lymphocytes. Examples of the literature in this area including Knuth et al., Proc. Natl. Acad. Sci. USA 86: 2804-2802 (1984); Mukherji et al., J. Exp. Med. 158: 240 (1983); Herin et all, Int. J. Canc. 39: 390-396 (1987); Topalian et al, J. Clin. Oncol 6: 839-853 (1988). Stable cytolytic T cell clones have been derived from MLTC responder cells, and these clones are specific for the tumor cells. See Mukherji et al., supra, Herin et all, supra, Knuth et al., supra. The antigens recognized on tumor cells by these autologous CTLs do not appear to represent a cultural artifact, since they are found on tumor cells in vivo. Topalian et al., supra; Degiovanni et al., Eur. J. Immunol. 20: 1865-1868 (1990). These observations, coupled with the techniques used herein to isolate the genes for specific murine tumor rejection antigen precursors, have led to the isolation of nucleic acid sequences coding for tumor rejection antigen precursors of TRAs presented on human tumors. It is now possible to isolate the nucleic acid sequences which code for tumor rejection antigen precursors, including, but not being limited to those most characteristic of a particular tumor, with ramifications that are described infra.

Brief Summary Text (17):
In U.S. Pat. No. 5,487,974 and incorporated by reference herein, tyrosinase is described as a tumor rejection antigen precursor. This is a well known molecule as per Kwon, U.S. Pat. No. 4,898,814. This reference discloses that a molecule which is produced by some normal cells (e.g., melanocytes), is processed in tumor cells to yield a tumor rejection antigen that is presented by HLA-A2 molecules. The peptide presented thereby is described in U.S. Application Ser. No. 08/057,714, filed Apr. 28, 1993, now abandoned also incorporated by reference. Additional tyrosinase derived peptides presented by HLA molecules are set forth in Ser. No. 08/203,054, now U.S. Pat. No. 5,530,096 and Ser. No. 08/233,305 now U.S. Pat. No. 5,519,117 filed Feb. 28, 1994 and Apr. 26, 1994 and are incorporated by reference.

Brief Summary Text (18):
Other peptides which are TRAs are described in additional patent applications. U.S. patent application Ser. No. 08/195,186, now U.S. Pat. No. 5,558,995 filed Feb. 14, 1994, and incorporated by reference herein, sets forth three peptides, which are derived from MAGE-1 and which complex with HLA-Cw* 1601. Ser. No. 08/196,630, now abandoned filed Feb. 15, 1994, discloses an unrelated tumor rejection antigen precursor, the so-called "BAGE" gene, and peptides derived therefrom, which are processed and then presented by HLA-Cw* 1601. Additional coding sequences for a tumor rejection antigen precursor referred to as Melan-A are set forth in Ser. No. 08/032,978, now U.S. Pat. No. 5,620,886 filed Mar. 18, 1993 and incorporated by reference. A more extended sequence for this gene is set forth in Ser. No. 08/272,351, now abandoned filed Jul. 8, 1994 incorporated by reference. In Ser. No. 08/96,039, filed Jul. 22, 1993, now abandoned the sequence of tumor rejection antigen precursor GAGE is set forth, and is incorporated by reference.

Brief Summary Text (19):
A series of peptides which provoke lysis by cytolytic T cells when presented by MHC molecules are set forth in Ser. No. 08/217,186, now U.S. Pat. No. 5,585,461 Ser. No. 08/217,188, now U.S. Pat. No. 5,554,724 and Ser. No. 08/217,187, now U.S. Pat. No. 5,554,506 all filed on Mar. 24, 1994, and all of which are incorporated by reference herein. The first of these applications discloses MAGE-3 derived peptides presented by HLA-A2. Five peptides are of interest. The second application presents 11 sequences derived from MAGE-2, believed to complex with HLA-A2.1 molecules. The last of these applications discloses two additional peptides derived from MAGE-3 which complex to HLA-A2. Ser. No. 08/190,411, now U.S. Pat. No. 5,541,104 filed Apr. 1, 1994 and incorporated by reference, sets forth three peptides derived from MAGE-1, which are immunogenic in that they provoke production of antibodies in a host animal to which they have been administered. Ser. No. 08/253,503, now U.S. Pat. No. 5,589,334 filed Jun. 3, 1994 and incorporated by reference, teaches a further tumor rejection antigen precursor gene and a peptide, derived therefrom, which is presented by HLA-B44 molecules. Further in the application of Coulie, Ikeda and Boon-Falleur, Ser. No. 08/316,231 now U.S. Pat. No. 5,830,753 incorporated by references, a sequence coding for a tumor rejection antigen precursor known as DAGE is set forth. DAGE is found almost universally on tumor cells, and only on testis cells with respect to normal cell expression. This makes it especially useful for cancer diagnosis and in the applications disclosed herein. The above listing should not be presumed to be exhaustive of the TRAP and TRA literature, but is presented to show its diversity and the fact that these materials not only provoke T cell proliferation, but also stimulate production of antibodies. It is well known that antibody producing cells can be used as

a source to produce hybridomas, which in turn produce monoclonal antibodies. Thus, when the term "antibodies" is used herein, it encompasses both polyclonal and monoclonal antibodies.

Detailed Description Text (27):
Following completion of the protocols of Example 1, patients received systemic pretreatment with GM-CSF, at 75 .mu.g per day, subcutaneously. Injection schedules are set forth in Tables 1 and 2, infra.

Detailed Description Text (37):
Peptides, as set forth in Table 3, were used. Three of the patients described supra, i.e., NW28, NW29 and NW30, were tested. All had measurable metastatic disease. The patients were injected with 100 ug of each peptide (200 ul final volume), intradermally, at disease free sites at least 10 cm apart. Preferred sites for peptide injection were the periumbilical region, the front parts of the thigh, and the medial part of the forearms. Injections were repeated, weekly, four times. During the fourth immunization cycle, GM-CSF was injected (75 ug, subcutaneously), for daily injections. The GM-CSF injections began three days before peptide immunization, and continued until two days after injection.

Detailed Description Text (49):
The amount of immunogen, e.g., TRAP or TRA used will vary, depending upon the purpose of the immunization and the subject to which it is administered. For example, in the case of generating murine antibodies which can then be used, e.g., to diagnose for the presence of cancer cells presenting a TRA, the amount of protein or peptide may be less than that used in a course of in vivo therapy, such as that described in the example, supra. In general, a preferred dose can range from about 1 ug to about 750 ug of protein or peptide per dose. In a preferred embodiment, the range is from about 10 ug to about 500 ug. Most preferably, anywhere from about 30 ug to about 300 ug per dose may be used. Of course, in the context of the therapeutic aspect of the invention, the investigator will modify the dose, as a six month old infant will require dosing different from a full grown man, e.g. The mode of administration may vary, with preferred forms being oral, subcutaneous, intramuscular, intravenous and intraperitoneal administration.

Detailed Description Text (50):
The choice of immunogen, e.g., TRAP or TRA protein or peptide in the composition will depend upon parameters determinable by the artisan. It is art recognized, for example, that different TRAs are presented by the various MHC molecules. As such, if a subject is typed, using well known techniques, as presenting HLA-A2 molecules on the surface of tumor cells, one will use a TRA presented by HLA-A2 molecules rather than one presented by, e.g., HLA-Cw* 1601, HLA-A1, HLA-B24, HLA-B8, HLA-B44, HLA-Cw6, etc. All of these MHC molecules are known to present tumor rejection antigens. Similarly, using techniques such as polymerase chain reaction ("PCR"), lysis studies, and other assay methodologies which are well known in the art, one can determine which tumor rejection antigen precursor gene or genes are being expressed by a subject patient. This will lead to the decision as to what protein or peptide to use. Again, by way of example, if a subject's tumor cells are expressing MAGE-3 but not MAGE-1, the peptide used in immunization should be derived from MAGE-3, and not MAGE-1.

Detailed Description Text (55):
The amounts of immunogen and GM-CSF used may vary, depending upon the particular application; however, a single dose of immunogen is preferably anywhere from about 10 ug up to about 5000 ug, more preferably from about 50 ug to about 2500 ug, most preferably about 100 ug to about 1000 ug. For GM-CSF, a dose of 10 ug to about 100 ug per dose is preferred. Modes of administration possible include intradermal, subcutaneous, and intravenous administration, implantation in the form of a time release formulation, etc. Any and all forms of administration known to the art are encompassed herein.

Detailed Description Text (56):
While the preferred molecules discussed herein are referred to as "tumor" rejection antigens and "tumor" rejection antigen precursors, it is intended that their use, in a therapeutic and also a diagnostic context, extends beyond cancer per se. The art is familiar with pathological conditions, such as diaplastic nevis, which are not cancer per se, but where the cells of the afflicted individuals are in fact characterized by transformation. Any and all such conditions are within the intended ambit of the invention.

CLAIMS:

1. An immunogenic composition consisting of:

(a) at least one peptide molecule, said at least one peptide molecule consisting of an amino acid sequence for a tumor rejection antigen, where

said peptide molecule binds to an MHC molecule on a cell surface to provide a peptide--MHC complex; and

(b) an amount of granulocyte macrophage colony stimulation factor sufficient to stimulate an antigen specific, CD8.sup.+ cytolytic T cell response against said peptide--MHC complex.

4. A method for generating an antigen specific, CD8.sup.+ cytolytic T cell response in a patient in need of an antigen specific, CD8.sup.+ cytolytic T cell response comprising administering separately to said patient:

(a) at least one peptide molecule consisting of an amino acid sequence for a tumor rejection antigen, wherein said peptide molecule binds to an MHC molecule on a cell surface to provide a peptide--MHC complex; and

(b) an amount of granulocyte macrophage colony stimulating factor (GM-CSF) sufficient to stimulate an antigen specific, CD8.sup.+ cytolytic T cell response directed against said peptide--MHC complex.

14. A kit useful for generating an antigen specific, CD8.sup.+ cytolytic T cell response against a tumor rejection antigen, consisting of separate portions of:

(a) at least one peptide molecule consisting of an amino acid sequence for a tumor rejection antigen (TRA), where said peptide molecule binds to an MHC molecule on a cell surface to provide a peptide--MHC complex; and

(b) an amount of granulocyte macrophage colony stimulation factor sufficient to stimulate an antigen specific, CD8.sup.+ cytolytic T cell response directed against said peptide--MHC complex.

17. A method for generating an antigen specific, CD8.sup.+ cytolytic T cell response to a patient in need of an antigen specific, CD8.sup.+ cytolytic T cell response, wherein said patient has a known HLA type, comprising administering to said patient, in sequential order:

(a) one of (i) a tumor rejection antigen (TRA) or (ii) a tumor rejection antigen precursor (TRAP) wherein said TRAP is processed in vivo to a TRA, which binds to said HLA; and (iii) an amount of GM-CSF effective for stimulating a first, antigen specific CD8.sup.+ cytolytic T cell response to said TRA; followed by

(b) an additional amount of said TRA of (a)(i) or TRAP of (a)(ii); and

(c) an additional amount of GM-CSF effective for stimulating a second, antigen specific CD8.sup.+ cytolytic T cell response to said TRA.

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L2: Entry 1 of 1 File: USPT Jun 19, 2001

DOCUMENT-IDENTIFIER: US 6248332 B1
TITLE: Targeted immunostimulation with bispecific reagents

US PATENT NO. (1):
6248332

Detailed Description Text (2):
An optimal antibody response to a thymus-dependent antigen requires that the B cell obtain help from a CD4+ helper T cell. The B cell is uniquely designed to accomplish this in that it contains antigen-specific immunoglobulin on its surface which allows it to bind, internalize and process antigen for presentation very efficiently. Other antigen presenting cells, such as the macrophage and dendritic cell, lack antigen-specific receptors, and therefore also lack this highly efficient mechanism for processing and presenting antigen. However, the apparent requirement for adjuvants when administering vaccines suggests a need for an antigen presenting cell in addition to the B cell. Also, it appears that antigen presentation by resting B cells to resting T cells does not lead to a T cell activation, but rather to T cell tolerance (Eynon et al. (1992) J. Exp. Med. 175:131). This is due to the failure of the resting B cell to deliver all the signals required for activation of the resting T cell. On the other hand, it appears that induction of T cell tolerance by the resting B cell could be averted if the resting T cell first responds to antigen on the antigen presenting cell such as the macrophage or dendritic cell (Parker et al. (1991) FASEB J. 5:2777). This implies that in the naive individual, the resting T cell must first interact with a macrophage or dendritic cell before interacting with the resting B cell.

Detailed Description Text (3):
These considerations have lead to the conclusion that the optimal immunogen requires two major components: antigen which can be recognized by the antigen-specific B cell; and a component which directs antigen for efficient processing and presentation to an antigen presenting cell other than the resting B cell (Parker et al., ibid.; Germain (1991) Nature 353:605). Attaching antigens to anti-Fc receptor antibodies satisfies these criteria since antigen directed to Fc receptors on the macrophage enhances antigen presentation at least 100 fold (Immunol. Today (1985) 6:245). Studies in vivo support the efficacy of such a vaccine. For example, a substantial increase in antibody production has been observed following immunization of mice with bispecific antibody which directed antigen to MHC class II or Fc.gamma.RII (Snider et al. (1990) (J. Exp. Med. 171:1957-1963). In addition, the requirement for adjuvant was eliminated. The ability to use substantially lower doses of immunogens is especially valuable when administering immunogens such as allergens that are potentially toxic at higher doses. Tolerance against some allergens can be obtained by repeated low dose administration of the allergen. Tolerance may result from the production of IgG against the allergen, which competes with allergen-specific IgE, removing the allergen so that it will not interact with IgE-coated mast cells. Allergen-anti-Fc receptor conjugates have the potential to both reduce the amount of allergen administered, thereby further reducing toxicity, and, at the same time, increase the production of allergen-specific IgG.

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End of Result Set

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L18: Entry 2 of 2 File: USPT Aug 10, 1999

DOCUMENT-IDENTIFIER: US 5935568 A
TITLE: Gene therapy for effector cell regulation

Abstract Text (1):
The present invention provides a nucleic acid-based therapeutic composition to treat an animal with disease by controlling the activity of effector cells, including T cells, macrophages, monocytes and/or natural killer cells, in the animal. Therapeutic compositions of the present invention include superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules, depending upon the disease being treated. The present invention also relates to an adjuvant for use with nucleic acid-based vaccines. Adjuvant compositions of the present invention include an immunogen combined with superantigen-encoding nucleic acid molecules, either in the presence or absence of a cytokine-encoding nucleic acid molecule and/or chemokine-encoding nucleic acid molecules.

Drawing Description Text (9):
FIG. 8 illustrates the response of V.beta.3+ T cells to intramuscular injection of a superantigen-encoding DNA plasmid.

Detailed Description Text (47):
Another preferred delivery vehicle comprises a recombinant cell vaccine. Preferred recombinant cell vaccines of the present invention include tumor vaccines, in which allogeneic (i.e., cells derived from a source other than a patient, but that are histiotype compatible with the patient) or autologous (i.e., cells isolated from a patient) tumor cells are transfected with recombinant molecules contained in a therapeutic composition, irradiated and administered to a patient by, for example, intradermal, intravenous or subcutaneous injection. Therapeutic compositions to be administered by tumor cell vaccine, include recombinant molecules of the present invention without carrier. Tumor cell vaccine treatment is useful for the treatment of both tumor and metastatic cancer. Use of a tumor vaccine of the present invention is particular useful for treating metastatic cancer, including preventing metastatic disease, as well as, curing existing metastatic disease. Methods for developing and administering include those standard in the art (see for example, Dranoff et al., Proc. Natl. Acad. Sci. USA 90:3539-3543, 1993, which is incorporated herein by reference in its entirety).

Detailed Description Text (71):
Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference in its entirety). Oral delivery can be performed by complexing a therapeutic composition of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a therapeutic composition of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Detailed Description Text (81):
Suitable compounds to combine with an adjuvant of the present invention, to form an adjuvant composition (i.e., a vaccine composition useful as a preventative therapeutic reagent or a therapeutic remedy useful to alleviate a disease) of the present

invention, include any compound that is administered to an animal as an immunogen. As used herein, an immunogen of the present invention comprises a compound capable of eliciting an immune response in an animal. Preferably, an immunogen of the present invention is derived from a foreign agent including a pathogen. Also preferably, an immunogen of the present invention includes an allergen (organic or inorganic), tumor antigens and self-antigens.

Detailed Description Text (87):
An effective administration protocol (i.e., administering an adjuvant composition in an effective manner) comprises suitable dose parameters, and modes and times of administration that result in the treatment of an animal. Effective dose parameters and modes of administration can be determined using methods standard in the art for a particular adjuvant composition. Such methods include, for example: determination of side effects (i.e., toxicity) of an adjuvant composition; progression of a disease upon administration of an adjuvant composition; magnitude and/or duration of antibody response by an animal against an immunogen contained in an adjuvant composition; magnitude and/or duration of a cell mediated immune response in an animal against an adjuvant composition; similarity of an immune response to an adjuvant composition in different species of animals; and/or effect of breed (in non-human animals) or race (in humans) on responsiveness to an adjuvant composition. In particular, the effectiveness of dose parameters and modes of administration of an adjuvant composition of the present invention can be determined by assessing antibody production in vivo, skin test sensitivities in vivo, cytokine production in vitro, antigen-specific proliferation in vitro, cytotoxic T cell activity in vitro, reduction of tumor burden in vivo and/or reduction of infectious agent burden in vivo. Tests standard in the art can be used to determine antibody production (e.g., enzyme-linked immunoassays), skin test sensitivities (e.g., subcutaneous injection of an immunogen into a vaccinated animal to assess weal formation, induration and erythema), cytokine production (e.g., immunoassays using cytokine-specific antibodies or bio-assays using cytokine-dependent cell lines), antigen-specific proliferation (e.g., .sup.3 H-thymidine incorporation), cytotoxic T cell activity (e.g., measure release of .sup.51 Cr from target cells), reduction of tumor burden (e.g., measure size of a tumor) and/or reduction of infectious agent burden (e.g., obtaining, for example, viral titers, bacterial colony counts or parasite counts).

Detailed Description Text (97):
Adjuvant compositions are preferably delivered by intramuscular administration in the form of "naked" DNA molecules, such as disclosed herein. Preferably, an adjuvant composition of the present invention is delivered by intramuscular, intravenous, intraperitoneal and/or intraarterial injection, and/or injection directly into specific cellular locations (e.g., into a tumor). Preferred sites of intramuscular injections include caudal thigh muscle, back muscle and into the buttocks of a human.

Detailed Description Text (98):
Preferably, an adjuvant composition of the present invention comprises a suitable pharmaceutically acceptable carrier for delivering the composition intramuscularly. A preferred carrier for use with an adjuvant includes phosphate buffered saline, water, Ringer's solution, dextrose solution, Hank's balanced salt solution and normal saline. A more preferred carrier includes phosphate buffered saline and normal saline, with phosphate buffered saline being even more preferred.

Detailed Description Text (135):
B16 cells were transfected with either PCR.sub.3 -SEA.S DNA, PCR.sub.3 -SEB.S or PCR.sub.3 DNA lacking insert (mock) using the method described in Example 2. The cells were then irradiated at 12,000 Rads. About 10.sup.6 irradiated cells were then injected subcutaneously into C57B16/J mice. Three weeks later, the mice were sacrificed and their spleen mononuclear cells harvested. Mononuclear cells isolated from the spleen cells were then restimulated in vitro with irradiated, non-transfected wild type B16 cells for 6 days in media with interleukin-2 (IL-2). The spleen cells were then added in decreasing numbers to about 5.times.10.sup.3 of .sup.51 Cr-labeled wild type (non-transfected) B16 cells in a standard chromium release assay for CTL activity. After 4 hours, the supernatants were harvested and the percentage of specific lysis of the target B16 melanoma cells was quantitated.

Detailed Description Text (162):
Four groups of mice B10.BR (2-3 mice per group) were prepared as follows. Group (1) consisted of untreated mice (control mice). Group (2) consisted of mice injected intraperitoneally with 100 ng of recombinant SEA (rSEA) protein. Group (3) consisted of mice injected intramuscularly with 100 .mu.g of PCR.sub.3 -SEA.S DNA (50 .mu.g per leg,

total of 100 .mu.g/mouse). Group (4) consisted of mice injected intramuscularly with 100 .mu.g PCR.sub.3 (no insert; mock) DNA (50 .mu.g per leg, total of 100 .mu.g/mouse). The DNA samples were prepared by diluting 100 .mu.l of a solution containing 100 .mu.g of DNA 50:50 (v:v) in sterile PBS prior to injection. The rSEA protein was purified from cultures of E. coli cells transformed with the recombinant molecule PKK223 (obtained from Dr. John Kappler) encoding the truncated SEA.S protein lacking a leader sequence.

Detailed Description Text (165):
The results are shown in FIG. 8 and indicate that the percentage of CD4+, V.beta.3+ T cells declined rapidly in PBMC of mice that received intramuscular injections with PCR.sub.3 -SEA.S DNA, but not in mice mock injected with mock DNA. The percentages of V.beta.8+ cells was not affected. This result is predicted since SEA protein does not bind mouse V.beta.8+ T cells. The decline of the percentage of CD4+, V.beta.3+ T cells occurred as rapidly as in mice injected with the recombinant SEA protein (rSEA). The deletion, however, observed over the next 2 months in mice injected with PCR.sub.3 -SEA.S DNA was longer lasting and was more pronounced than the deletion induced by injection of SEA.S protein. In addition, injection of as little as 2 .mu.g PCR.sub.3 -SEA.S DNA also induced deletion of V.beta.3+ T cells. Thus, intramuscular injection of DNA encoding superantigens represents a potent and non-toxic approach to the deletion or suppression of potentially harmful (e.g., autoreactive T cells) T cells.

Detailed Description Text (171):
Separate groups of 4 CB6 F1 mice per group were injected twice with the following mixtures of DNA: (1) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -MIP-1.beta.; (2) about 100 .mu.g PCR.sub.3 -OVA+about 50 .mu.g PCR.sub.3 -SEB (described in Example 1)+PCR.sub.3 -GM-CSF (described in Example 1); (3) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -RANTES; (4) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -SEB; (5) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -GM-CSF; or (6) about 100 .mu.g PCR.sub.3 -OVA alone. Control samples were also prepared which included 6 non-injected, syngeneic mice. The DNA was diluted to a final concentration of 0.5 mg/ml in sterile phosphate buffered saline (PBS) prior to injection. The mice were injected intramuscularly, bilaterally in their quadriceps muscles (about 100 .mu.g of DNA per quadricep).

Detailed Description Text (176):
Separate groups of 4 CB6 F1 mice per group were injected twice, intramuscularly (on day 1 and day 21), with the following mixtures of DNA: (1) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -MIP-1.beta.; (2) about 100 .mu.g PCR.sub.3 -OVA+about 50 .mu.g PCR.sub.3 -SEB+PCR.sub.3 -GM-CSF; (3) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -RANTES; (4) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -SEB; (5) about 100 .mu.g PCR.sub.3 -OVA+about 100 .mu.g PCR.sub.3 -GM-CSF; or (6) about 100 .mu.g PCR.sub.3 -OVA alone. Control samples were also prepared as above.

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DOCUMENT-IDENTIFIER: US 6406689 B1
TITLE: Compositions and methods for treatment of tumors and metastatic diseases

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Abstract Text (1):
Compositions and methods are provided which can be utilized in active immunization as a prophylactic treatment or a therapeutic treatment for tumors. The compositions are employed as injectable tumor vaccines or as preparations for intratumoral administration and are capable of stimulating immune responses to specific tumor antigens. The tumor vaccines are composed of an antigenic cellular material including a plurality of inactivated tumor cells or tumor cell portions, a depot material, and an immunostimulant adsorbed to the depot material. The depot material with absorbed immunostimulant is mixed with the tumor cells or tumor cell portions to form the vaccine compositions. The preparations for intratumoral administration include the depot material adsorbed immunostimulant without the antigenic cellular material. The immunostimulant adsorbed to the depot material permits release of biologically active quantities of the immunostimulant over a period of time rather than all at once.

Brief Summary Text (10):
Adjuvants. The natural ability of an antigen to induce an immune response can be modified, and in particular enhanced, by altering or by mixing it with another substance. The procedure or the substance used to enhance immune responses is called an adjuvant. At least three classes of adjuvants have been used for a long time; these are mineral oil emulsions, aluminum compounds, and surface active materials such as saponin, lysolecithin, retinal, Quil A.RTM., some liposomes, and pluronic polymer formulations. See, for example, Fundamental Immunology, edited by William E. Paul, at p. 1008, Raven Press, New York (this book will hereinafter be referred to as "Fundamental Immunology"). Aluminum adjuvants used alone or in combination include aluminum hydroxide gel, aluminum phosphate, aluminum sulphate, and alums comprising ammonium alum (such as (NH.sub.4).sub.2 SO.sub.4.Al.sub.2 (SO.sub.4).sub.3) and potassium alum. Aluminum hydroxide (hereinafter "AL") is one of the older adjuvants and it is considered so safe that it has been applied in bacterial and viral vaccines administered to billions of people around the world. Calcium phosphate gel (hereinafter "CP") has similar properties and is also used in vaccines. Both substances are available in pharmaceutical qualities in most countries worldwide. Techniques for preparing adjuvant-antigen preparations for injection are well known in the art. See, for example, Terry M. Phillips, Analytical Techniques in Immunochemistry, pp. 307-10, Marcel Dekker, New York, 1992.

Brief Summary Text (11):
Other adjuvants include complete Freund's adjuvant (a water-in-oil emulsion in which killed, dried, mycobacteria--usually M tuberculosis--are suspended in the oil phase); incomplete Freund's adjuvant (analogous to the complete Freund's adjuvant with no mycobacteria); ISCOM (or immune stimulating complex, comprising lipophilic particles formed by the spontaneous association of cholesterol, phospholipid and the saponin Quil A.RTM.); lipopolysaccharide (complex molecules consisting of a lipid core--lipid A--with a polysaccharide side chain that are components of certain bacilli, Lipid A is incorporated into the outer membrane of the bacterium and the polysaccharide projects extracellularly. Their adjuvant potency is associated with lipid A; they are also mitogenic for murine B lymphocytes); and mycobacterial adjuvants (whole, heat killed, dried, mycobacteria--such as M. tuberculosis, M. avium, M. phlei, and M. smegmatis) that, when suspended in mineral oil and emulsifier, have adjuvant activity with respect to any antigen given with them. Extracts of some mycobacteria, e.g., mycobacterial

peptidoglycolipids have similar adjuvant activities. See, for example, Dictionary of Immunology at pp. 3, 7, 46, 94, 97, 105, and 116; R. B. Luftig, Microbiology and Immunology, pp. 228-29, Lippincott-Raven Publishers, Philadelphia 1998. Microbial adjuvants include Corynebacterium parvum and Bordetella pertussis. See, for example, Handbook of Immunology at 115-16. Use of controlled-release preparations and materials with adjuvant activity and possible sites of action have been described in Fundamental Immunology at pp. 1007-09.

Brief Summary Text (12):
Mineral carriers such as aluminum hydroxide, potassium ammonium sulphate, and potassium aluminum sulphate adsorb the antigen on their surface. These common adjuvants have been used at a 0.1% concentration with up to 1 mg protein antigen in 1 ml administered to animals at doses of 0.2-0.5 ml/(kg body weight). See Miroslav Ferencik, Handbook of Immunochemistry, p. 115, Chapman & Hall 1993 (this book will hereinafter be referred to as "Handbook of Immunochemistry"). Although Freund's adjuvant is toxic and not used for immunization of human beings, mineral adjuvants such as aluminum hydroxide are common in human medicine. Id. at 116. In addition to alum, other adjuvants in the group of inert carriers include bentonite, latex, and acrylic particles. See Fundamental Immunology at 1008.

Brief Summary Text (13):
Combinations of adjuvants can also have adjuvant properties. For example, it has been shown that the combination of saponin and muramyl dipeptide in a squalene in water emulsion is superior to alum as an adjuvant for inducing certain responses in mice. R. Bomford, M. Stapleton, S. Wilson, A. McKnight, and T. Andronova, The control of the antibody isotype responses to recombinant human immunodeficiency virus gp120 antigen by adjuvants, AIDS Res. Hum. Retroviruses Vol. 8(1992) pp. 1765 et seq. These adjuvants are complemented by new adjuvants that have been developed during the last fifteen years. See, for example, Anthony C. Allison, The Role of cytokines in the Action of Immunological Adjuvants, in Vaccine Design. The Role of cytokine Networks, edited by Gregory Gregoriadis and Brenda McCormack, NATO ASI Series A: Life Sciences Vol 293, pp. 1-9, Plenum Press, New York 1997 (this book will hereinafter be referred to as "Vaccine Design"); Immunology at p. 116; H. Snippe, I. M. Fernandez and C. A. Kraaijeveld, Adjuvant Directed Immune Specificity at the Epitope Level. Implicationsfor Vaccine Development. A Model Study Using Semliki Forest Virus Infection of Mice, in Vaccine Design at pp. 155-73.

Brief Summary Text (14):
An adjuvant can be administered prior to, simultaneously with, or following the administration of the antigen. Antibody production enhancement caused by adjuvants is not fully understood. However, adjuvant properties that may exist either alone or in various combinations and which permit a substance or formulation to be described as adjuvant active have been defined. See, for example, J. C. Cox and A. R. Coulter, Adjuvants--A classification and review of their modes of action, Vaccine Vol. 15(1981) pp. 248 et seq.; John Cox, Alan Coulter, Rod Macfarlan, Lorraine Beezum, John Bates, Tuen-Yee Wong and Debbie Drane, Development of an Influenza-ISCOM.TM. Vaccine, in Vaccine Design at pp. 33-49. One of these properties is depot generation, whereby the vaccine is retained near the dose site to give short term trickle release or a longer term pulsed release. Id. at p. 34. The term "depot" will hereinafter be used to refer to an adjuvant or to the combination of an adjuvant and at least one immunostimulating substance that is administered with antigenic material for enhancing the immune response.

Brief Summary Text (21):
Immunomodulators, of ten contained in adjuvants, induce the production of cytokines, thus enhancing immune responses. Examples are muramyl peptides, lipopolysaccharides and derivatives, and certain cationic detergents. See, for example, Anthony C. Allison, The Role of Cytokines in the Action of Immunological Adjuvants, in Vaccine Design at pp. 1-9. Interleukin active domains or the corresponding synthetic peptides could in fact be potent adjuvants, as shown for a region of an IL-1. See, for example, Aldo Tagliabue and Diana Boraschi, Interleukin 1 and Its Synthetic Peptide 163-171 as Vaccine Adjuvants, in Vaccine Design at pp. 167-73.

Brief Summary Text (34):
Active specific immunotherapy approaches to the treatment of tumors have been widely investigated during recent years. Numerous studies involving the vaccination of patients with their own inactivated tumor cells have been reported. These studies have demonstrated that inclusion of an adjuvant is necessary to stimulate the patient's immune system against the autologous, or derived from self, tumor cells. For example,

methods utilizing the particulate adjuvant, Bacillus Calmette-Guerin (BCG) cells, administered systemically or mixed with the patient's own tumor cells have been shown to induce tumor-specific immunity in laboratory animals. Peters, L. C., Brandhorst, J. S., Hanna Jr., M. G., Preparation of Immuno-Therapeutic Autologous Tumor Cell Vaccines from Solid Tumors; Cancer Res. 39: 1353-1360 (1979).

Brief Summary Text (35):
This approach has been investigated with different tumor types. The administration of inactivated tumor cells in a mixture including a bacterial adjuvant resulted in significantly improved survival rates in patients with metastasized renal cell carcinoma. Tallberg, T., Tykka, H., Specific Active Immunotherapy in Advanced Renal Cell Carcinoma: A Clinical Long-Term Follow-Up Study; World J Urology 3: 234-44 (1986). A statistically significant increase in patient survival rates was also achieved with the use of Newcastle Disease Virus (NDV) as an adjuvant in clinical trials for the treatment of malignant melanoma. Cassel, W. A., Murray, D. R., Phillip, H. S., A Phase II Study on The Post Surgical Management of Stage II Malignant Melanoma With a Newcastle Disease Virus Oncolysate, Cancer 52: 856-60 (1983). A similar protocol employed in animal tumor models demonstrated that administration of a vaccine prepared by irradiating ESb tumor cells infected with the apathogenic NDV induced permanent T-cell immunity towards antigens of the introduced tumor type. Schirrrnacher, V., Ahlert, T., Heicappel, R., Appelhans, B., Von Hoegen, P., Successful Application of Non-Oncogenic Viruses for Antimetastatic Cancer Immunotherapy; Cancer Reviews 5: 19-49 (1986); Schirrmacher, V., Immunity and Metastasis: In Situ Activation of Protective T Cells by Virus-Modified Cancer Vaccines; Cancer Survey 13: 139-154 (1992). Moreover, the immunity was transferable to other animals by adoptive transfer of lymphocytes. Schirrmacher, V. Von Hoegen, P. Griesbach, A., Zangemeister-Wittke, U., Specific Eradication of Micrometastasis by Transfer of Tumor-Immune T-Cells From MHC-Congenic Mice; Cancer Immunol. and Immunother. 32: 373-81 (1991).

Brief Summary Text (36):
NDV, however, is not a conventional adjuvant. During incubation with tumor cells the fowl-pathogenic and human apathogenic virus binds to the tumor cells and the virus membrane gets integrated into the tumor cells membrane which by this gets a certain degree of xenogenization. In addition, the virus membrane contains components (hemagglutinins) to which human (and animal) cells (lymphocytes) can bind. It is anticipated that the immune system is stimulated by the xenogenization of the tumor cell membranes altered by the interaction of the virus membrane. In addition, lymphocytes can bind to the hemagglutinin moiety the tumor cells have acquired from the virus and become stimulated upon binding. This might also render the otherwise non-immunogenic parts of the tumor cells more immunogenic.

Brief Summary Text (37):
Regarding the use of adjuvants in cancer treatment, one may think of using a substance with well-known adjuvant properties such as aluminum hydroxide. This substance is used in bacterial and viral vaccines for inducing humoral immunity, which is antibody-based immunity. However, it is acknowledged that the induction of humoral immunity responses is or can be counterproductive--depending on the type of tumor--in tumor therapy. It has been shown that antibodies to tumor antigens might mask the tumor antigens and thus protect the tumor from the desired aggression by T-lymphocytes and other immune system cells. Due to this known property of inducing immune responses that might even protect the tumor from the attack of the cellular immune system, most researchers have ignored aluminum hydroxide and have not investigated its properties as an adjuvant for tumor vaccines. Those who have investigated these properties in animal experiments have clearly shown that aluminum hydroxide in fact does not have adjuvant activity at all. See, B. E. Souberbielle, B. C. Knight, W. J. Morrow, D. Darling, M. Fraziano, J. B. Marriott, S. Cookson, F. Farzaneh, and A. G. Dalgleish, Comparison of IL-2 and IL-4-transfected B16-F10 cells with a novel oil-microemulsion adjuvant for B16-F10 whole cell tumor vaccine, Gene Therapy Vol. 3 (1996) 853-858. Consistently with this knowledge, some researchers have ceased using aluminum hydroxide in experimental tumor vaccination. In addition, it has been shown that aluminum hydroxide does not act as an adjuvant when injected together with tumor cells or when injected alone. This knowledge is supplemented by the already reported property of aluminum hydroxide as an adjuvant only for soluble protein or for carbohydrate antigens, not for cells.

Brief Summary Text (40):
Cytokines have been reported as having generally potent effects on the development of the immune response to tumors and as eliciting a response capable of rejecting tumors. See, for example, A. McAdam, B. Pulanski, S. Harkins, E. Hutter, J. Frelinger, and E. Lord, Coexpression of IL-2 and .gamma.-IFN Enhances Tumor Immunity, in Immunotherapy of

Cancer With Vaccines at p. 349. Cytokines have also been used in vaccines for humoral immune responses. See, for example Dragana Jankovic, Patricia Caspar, Martin Zweig, Maria Garcia-Moll, Stephen D. Showalter, Frederick R. Vogel, and Alan Sher, Adsorption to Aluminum Hydroxide Promotes the Activity of Il-12 as an Adjuvant for Antibody as Well as Type 1 Cytokine Responses to HIV-1 gp 120, The Journal of Immunology Vol. 159 (1997) pp. 2409-17, at p. 2412. Although cytokines may play crucial roles in therapeutic vaccines for cancer treatment, these observations require a call for caution "because cytokines have as much potential to stimulate tumor growth as to retard it, and many cytokines effectively suppress immune responses under some conditions"; it is further acknowledged that these "complexities can only be unraveled by additional animal studies and direct testing in humans of promising candidate cytokines." John A. Sogn, John F. Finerty, Anne K. Heath, Grace L. C. Shen, and Faye C. Austin, Cancer Vaccines: The Perspective of the Cancer Immunology Branch, NCI, in Immunotherapy of Cancer With Vaccines, at p. 327.

Brief Summary Text (50):
It is known that Granulocyte-Macrophage Colony-Stimulating-Factor (hereinafter referred to as "GM-CSF") plays an essential role in induction of tumor immunity. GM-CSF is a cytokine that is made by a number of cells, including lymphocytes and it is necessary for differentiation of lineage-specific stem cells. B16 mouse melanoma cells which had been transduced with the genes for both IL-2 and GM-CSF induced stronger immunity to this tumor than any other cytokine-gene transfected Btumor cell. Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D., Mulligan, R. C., Vaccination With Irradiated Tumor Cells Engineered to Secrete Murine Granulocyte-Macrophage Colony-Stimulating-Factor Stimulates Potent, Specific, and Long-Lasting Anti-Tumor Immunity; Proc. Natl. Acad. Sci. USA 90: 3539-3543 (1993). The underlying molecular and cellular events are not entirely understood. It could be that GM-CSF released from the tumor cells is actively recruiting cells that are essential for primary immune responses, such as dendritic cells, and attracting these to the injection site or to the tumor cell itself. The dendritic cells then may take up antigen for presentation to T-lymphocytes attracted by cytokines released by the dendritic or other cells and/or by IL-2 released by the transfected tumor cells. Alternatively, or in addition, the dendritic cells may carry the tumor antigens to the regional lymph nodes and thereby expose the antigens to other immune system cells. It would appear that the natural immune response is more closely mimicked with vaccines incorporating the local release of two, or more, cytokines than by vaccines incorporating the release of only one cytokine.

Brief Summary Text (52):
Despite the promising results with cytokine-gene-transfected cells in experimental animals, adaptation of these methods to patients faces several hurdles. As an initial matter, the technical difficulty and cost of generating sufficient quantities of gene-transfected tumor cells from a primary tumor specimen is significant. The tumor cells must be recovered from the tumor of which only a small specimen is usually available. The recovered tumor cells must be adapted to in vitro growth. This is a tedious and of ten unsuccessful procedure. The cells must be transfected in a procedure which has variable success with different tumor types and with cells of the same tumor type from different patients. See, V. W. Simons et al., Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by in vivo GM-CSF gene transfer, Cancer Research, Vol. 57 (1997) 1537-1546; R. Soiffer et al., Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulatingfactor generates potent antitumor immunity in patients with metastatic melanoma, Proceedings of the National Academy of Sciences, Vol. 95 (1998) pp. 13141-13146. (The two immediately proceeding articles will hereinafter be collectively referred to as "Irradiated cancer cells and GM-CSF secretion. ") Unlike tumor cells obtained from animal tumor cell lines, which are homogeneous, tumor cells recovered from a human primary cancer lesion represent an extremely heterogeneous population of cells differing in their genotypes and phenotypes. Efficiency of transfection in such a cell population can be expected to be much less than in the quasi-monoclonal animal tumor cell populations. The transfected tumor cells with the highest cytokine production rate must be identified and selected. Thus, the overall procedure is time- and cost-intensive and the results can be unpredictable.

Brief Summary Text (61):
An object of the present invention is the induction of immune responses to tumors. In particular, an object of the present invention is the design of a depot formulation including an immunostimulant such as a cytokine that is optimally suited as an adjuvant for the induction of cell-dependent cytotoxic immune responses to cellular antigens.

Brief Summary Text (71):
A preferred depot material is an aluminum-based depot such as aluminum hydroxide, although other inorganic materials such as calcium phosphate as well as other inorganic or organic particles which are capable of adsorbing proteins such as latex particles and ion exchangers can also be used as depot material, along with various combinations thereof. In the context of this invention, the cytokine adsorbed to the depot material is, in combination, an adjuvant.

Brief Summary Text (75):
The tumor vaccine inoculum can be injected to prevent proliferation and growth of various tumors such as melanoma, renal carcinoma, prostate carcinoma, colon carcinoma, pancreas carcinoma, and lung carcinoma, as well as B lymphoma. It should be understood that a particular vaccine composition will contain tumor cells that correspond to the particular tumor being treated. Thus, a vaccine composition for renal carcinoma will contain renal carcinoma cells or cellular portions thereof. The tumor vaccine inoculums can be injected into a patient subcutaneously and/or intradermally.

Drawing Description Text (33):
FIG. 31 is a graph showing IL-2 concentrations in peripheral blood as a function of time after subcutaneous administration of free IL-2; and

Drawing Description Text (34):
FIG. 32 is a graph showing IL-2 concentrations in peripheral blood as a function of time after subcutaneous administration of IL-2/depot combinations.

Detailed Description Text (14):
Inorganic particles and gels such as aluminum hydroxide (AL) are known for their capacity for adsorbing proteins and have been used in vaccines, e.g., tetanus vaccine prepared with aluminum hydroxide gel (Alhydrogel). Particles or gels made of calcium phosphate also have an adsorptive capacity for biomolecules and have been utilized in bacterial vaccines and as DNA carriers for transfection experiments. It is possible to adsorb or otherwise chemically bind immunostimulants to such particles or gels and in general to combine immunostimulants to adjuvants.

Detailed Description Text (25):
Aluminum hydroxide has an inherent adjuvant property and consistently with this property it has been used in bacterial and viral vaccines. Such vaccines are used to induce humoral immunity, which is antibody-mediated immunity. Aluminum hydroxide is known in particular for its ability to enhance this type of immune response. However, humoral immune responses have been proven counterproductive in tumor therapy. It is believed that this effect is due to antibodies to tumor antigens masking the tumor antigens themselves, thus protecting the tumor from the aggression by cytotoxic T-lymphocytes and other immune system cells. Consistently with this experience, some research has backed away from the use of aluminum hydroxide in experimental tumor vaccination. It has also been shown that aluminum hydroxide does not act as an adjuvant when it is injected together with inactivated tumor cells, and that animals treated with such vaccines die as fast or even faster than animals that were treated with inactivated tumor cells alone. Furthermore, experiments carried out in the context of this invention have shown that the administration of aluminum hydroxide alone to control groups provided no protection.

Detailed Description Text (26):
It is also known that aluminum hydroxide is an adjuvant only for soluble protein or for carbohydrate antigens, but not for antigenic material in the form of inactivated cells. However, it has been found in the present invention that aluminum hydroxide is optimally suited as a depot for cytokines. Accordingly, cytokines adsorbed to aluminum hydroxide are optimally suited as adjuvants for cell-dependent cytotoxic immune responses to cellular antigens.

Detailed Description Text (47):
Tumor induction can be performed at any location. For most of the vaccinations, tumor inductions were done subcutaneously. Tumor induction by intraperitoneal injection was also performed because it is the most effective way to induce a tumor, and mice get tumors faster than they do after subcutaneous induction. Because tumors develop so fast after intraperitoneal injection, the compositions and methods of this invention that cure mice with such intraperitoneally induced tumors provide very effective treatment.

Detailed Description Text (48):
Vaccination is much more effective by intraperitoneal injection than by subcutaneous

injection, and intramuscular injection is less effective than subcutaneous injection. However, intraperitoneal vaccination can generally not be performed in patients because it is painful and very dangerous. Therefore, most of the vaccinations were administered subcutaneously so that the administration conditions in animal experiments could be as similar as possible to those in future human trials.

Detailed Description Text (49):
In the second class of therapeutic experiments, intratumoral therapy was performed. The preparations were administered into comparatively large tumors in mice that had been previously injected subcutaneously with live tumor cells to locally induce tumor growth. When the tumors were palpable, usually after one or two weeks, the tumor masses were about 0.1 to 0.5 ml each, corresponding to approximately 10.sup.7 to 10.sup.8 tumor cells. In both prophylactic and therapeutic vaccination experiments, the number of vaccinations has to be adapted depending on the inherent immunogenicity of the tumors investigated. Thus, in the case of a tumor with some inherent immunogenicity like the RenCa, a single vaccination might be sufficient either to confer protection in prophylactic vaccination experiments or to cure the tumor bearing animals by therapeutic vaccination experiments. If the immunogenicity of the tumor is very low as in the B16 melanoma or if the tumor is non-immunogenic, many vaccinations are needed in order to induce protection by prophylactic vaccination. Several therapeutic vaccinations are required to cure animals from such tumors. But even then, a success can of ten only be achieved when the tumor induction dose is reduced by one or several orders of magnitude.

Detailed Description Text (59):
In the tables shown in the following examples, "n" stands for the number of individuals in each vaccination group. The forms of administration of the different agents to the individuals in each vaccination group are described by abbreviated terms that are standard in the art. These abbreviations include "iv" for intravenous, intravenously, intravenous injection, or intravenous administration, "i.p." for intraperitoneal, intraperitoneally, intraperitoneal injection or intraperitoneal administration, and "s.c." for subcutaneous, subcutaneously, subcutaneous injection or subcutaneous administration. "AL" stands for an aluminum hydroxide-based depot, "CP" stands for a calcium phosphate-based depot, and "Lip" stands for a liposome-based depot. The terms "10.sup.m B16" and "10.sup.m RenCa", where the exponent "m" is an integer, in the examples given below mean about 10.sup.m inactivated B16 cells and about 10.sup.m inactivated RenCa cells, respectively.

Detailed Description Text (76):
The calcium phosphate material used in the context of this invention was the material "Calcium Phosphate Adjuvant" produced by Superfos Biosector AS, Kvistgaard, Denmark, with 1.0% of calcium phosphate in the gel. Since the adsorption capacity of calcium phosphate is about 100 times lower than that of aluminum hydroxide, i e., 1.0 .mu.g IL-2/100 .mu.g CP, 100 fold larger amounts of calcium phosphate gel have to be used.

Detailed Description Text (85):
For a general practical reference manual on immunochemical techniques, see, for example, Terry M. Phillips, Analytical Techniques in Immunochemistry, Marcel Dekker, New York 1992, the disclosure of which is incorporated by reference herein. Immunization techniques for intradermal, intramuscular, intraperitoneal, and intravenous immunization that produce good results for most applications have been described. Id. at pp. 312-14.

Detailed Description Text (92):
This example discloses the most preferred composition in this invention. This composition comprises about 10.sup.6 inactivated RenCa cells, IL-2 at a dose of about 10 .mu.g adsorbed to about 10 .mu.g aluminum hydroxide depot. Variables such as amount of antigenic tumor cells in the form of inactivated tumor cells, type of adjuvant, the type and amount of cytokine that are administered in a therapeutic treatment, and antigenicity and immunogenicity of tumor cells are further analyzed in the following examples.

Detailed Description Text (110):
The influence of the number of inactivated tumor cells in the vaccination composition on survival is analyzed here in a B16 prophylactic study. Prophylactic vaccination was administered four times prior to day 0 (hereinafter "challenge day") when the mice were subcutaneously injected a dose of tumor inducing preparation. Specifically, the prophylactic vaccinations were administered four times (4.times.) on days 35, 28, 21, and 14 prior to the challenge day. The control group comprised 5 mice that were

subcutaneously injected medium on the same vaccination days that the mice in the vaccination groups were subcutaneously injected the corresponding vaccination preparation. Vaccination group 1 comprised 8 mice, and the vaccination preparation included about 10.sup.5 inactivated B116 cells, and about 10 .mu.g IL-2 adsorbed to about 10 .mu.g of aluminum hydroxide depot. Vaccination groups 2 and 3 comprised 6 mice each. The vaccination preparations in groups 2 and 3 differed from the preparation in group 1 in that the preparation in group 2 included about 10.sup.6 inactivated B16 cells and the preparation in group 3 included about 10.sup.7 inactivated B16 cells. Both preparations also contained about 10 .mu.g IL-2 adsorbed to about 10 .mu.g of aluminum hydroxide depot. Vaccination group 4 also comprised 6 mice, but the vaccination preparation only included about 10.sup.5 inactivated B16 cells, but no IL-2 or aluminum hydroxide. These characteristics are summarized in Table 4.

Detailed Description Text (141):
The influence of the IL-2 dose in the vaccination composition on survival is analyzed here in a B16 prophylactic study. Prophylactic vaccination was administered four times (4.times.) prior to day 0 (hereinafter "challenge day") when the mice were subcutaneously injected a dose of tumor inducing preparation. Specifically, the prophylactic vaccinations were administered 35, 28, 21, and 14 days prior to the challenge day. The control group comprised mice that were subcutaneously injected medium on the same vaccination days that the mice in the vaccination groups were subcutaneously injected the corresponding vaccination preparation. Vaccination group 1 comprised 6 mice, and the vaccination preparation included about 10.sup.5 inactivated B16 cells and about 30 .mu.g IL-2. Vaccination groups 2 and 3 comprised 7 and 8 mice, respectively. The vaccination preparations in groups 2 and 3 differed from the preparation in group 1 in that the preparation in group 2 included about 10 .mu.g IL-2 and the preparation in group 3 included about 10 .mu.g IL-2. Vaccination group 4 also comprised about 10.sup.5 inactivated B16 cells, but no IL-2 or aluminum hydroxide. Because none of the preparations in this example included an aluminum hydroxide depot, IL-2 at the indicated dosages was adsorbed to tumor cells, so that the cells themselves became the depot. These characteristics are summarized in Table 9.

Detailed Description Text (159):
The influence on survival of the cytokine depot in the vaccination composition is analyzed here in a B16 prophylactic study. Prophylactic vaccination was administered four times (4.times.) prior to day 0 (hereinafter "challenge day") when the mice were subcutaneously injected a dose of tumor inducing preparation. Specifically, the prophylactic vaccinations were administered 35, 28, 21, and 14 days prior to the challenge day. The control group comprised 5 mice that were subcutaneously injected medium on the same vaccination days that the mice in the vaccination groups were subcutaneously injected the corresponding vaccination preparation. Vaccination group 1 comprised 8 mice, and the vaccination preparation was 10.sup.5 B16 10 .mu.g IL-2 AL. Vaccination group 2 comprised 9 mice, and the vaccination preparation was 10.sup.5 B16 10 .mu.g IL-2 CP. The vaccination preparation for group 3 with 6 mice was 10.sup.5 B16, and group 4 was the control group with 5 mice. These characteristics are summarized in Table 12.

Detailed Description Text (162):
Significance (12.1) means that, although the survival rate of the mice treated with AL-containing vaccines is overall better as shown in FIG. 15, the difference is not significant. This is mainly due to the fact that the two corresponding curves practically have an identical course for survival rates between 100% and 50%, or for the time period 0-50 days. This parallelism in the first part of the two survival curves has a great effect on the statistical Significance and the two curves that appear so different from each other in FIG. 15 are not significantly different according to the statistical test used in the analysis of the results herein described. Significances (12.2), (12.3), (12.4), and (12.5) indicate that the positive effect on survival of compositions that include IL-2 adsorbed to an adsorbent as an adjuvant is an explanation that has to be accepted over alternative explanations that are based on compositions with no adjuvant and no cytokine. Significance (12.6) indicates that the positive effect on survival of compositions that include inactivated B16 cells is an explanation that has to be accepted over an alternative explanation that is based on compositions with no depot, no cytokine and no inactivated B16 cells.

Detailed Description Text (170):
Renal carcinoma was induced into mice via intraperitoneal injection of a lethal dose of vital carcinoma cells. Four days later, the mice were vaccinated with the compositions described in the first column of Table 13. Vaccination groups 1, 2 and 3 comprised five mice each and group 4 was the control group with four mice. Mice in groups 1-2 received

a therapeutic composition that included about 10 .mu.g G-CSF adsorbed to aluminum hydroxide each. G-CSF stands for granulocyte colony stimulating factor, which is a cytokine made chiefly by mononuclear phagocytes. In addition, the composition administered to mice in group 1 included about 10.sup.6 inactivated RenCa cells. The vaccination composition administered to mice in group 3 comprised about 10.sup.6 inactivated RenCa cells, but did not contain cytokine or any adjuvant, and the composition administered to the control group consisted of medium with no cytokine, RenCa cells or adjuvant. Recombinant human G-CSF is commercially available, but G-CSF is not a preferred cytokine for tumor vaccines.

Detailed Description Text (174):
Renal carcinoma was induced into mice via intraperitoneal injection of a lethal dose of vital carcinoma cells. Four days later, the mice were vaccinated with the compositions described in the second column of Table 14. Vaccination groups 1, 2, and 3 comprised six mice each, group 4 comprised five mice, and group 5 was the control group with four mice. Mice in groups 1-4 received a therapeutic composition that included about 10.sup.6 inactivated RenCa cells. The vaccination compositions administered to mice in groups 2 and 3 also included about 10 .mu.g GM-CSF adsorbed to aluminum hydroxide. GM-CSF stands for granulocyte-macrophage colony stimulating factor, which is a cytokine made by immune system cells that include lymphocytes. The vaccination compositions administered to mice in groups 1 and 3 also included about 3 .mu.g IL-4. The vaccination composition administered to mice in group 4 comprised about 10.sup.6 inactivated RenCa cells, but did not contain cytokine or depot, and the composition administered to the control group consisted of medium with no cytokine, RenCa cells or depot.

Detailed Description Text (182):
The influence on survival of transferred spleen cells in the vaccination composition is analyzed in a prophylactic study in which RenCa-immune spleen cells of a vaccinated donor were transferred to BALB/c mice ("adoptive transfer of spleen cells") in groups 1-3. Mice to which tumor was induced by an injection of 10.sup.5 vital RenCa cells in day -4 were therapeutically vaccinated by a single inoculation with the usual vaccine (about 10.sup.6 irradiated tumor cells and about 10 .mu.g IL-2 adsorbed to about 10 .mu.g AL). The spleen cells of the surviving animals were harvested 100 days after vaccination. The spleens of the animals were transferred into medium-containing sterile dishes and squeezed with a forceps until the cells were released from the spleen tissue. The cells were counted, and the cell suspension was adjusted to the required density, e.g, about 30.multidot.10.sup.6 cells per ml. Then the cells were injected intravenously into the animals (about 1 ml of the cell suspension containing the required amount of cells into each animal). Spleen cells of non-vaccinated donors, or "naive donors" were transferred to mice in group 4 the same day the mice in groups 1-3 received spleen cells of vaccinated donors. All the spleen cell transfers were carried out by intravenous injections. Seven days after the adoptive transfer, mice in groups 1-4 were intraperitoneally injected with vital RenCa tumor cells. This was day 0 or the "challenge day". Groups 1-4 comprised six mice each, and control group number 5 comprised four mice. Mice in groups 1 and 4 received about 30.multidot.10.sup.6 spleen cells, mice in group 2 received about 10.multidot.10.sup.6 spleen cells, and mice in group received about 3.multidot.10.sup.6 spleen cells. These characteristics are summarized in Table 16.

Detailed Description Text (202):
Each one of the five vaccination groups in this B16 prophylactic study comprised 5 mice. The vaccination preparation administered to groups 1-4 included about 10.sup.5 inactivated B16 cells. In addition, the vaccination preparations administered to groups 2 and 3 included about 10 .mu.g G-CSF, whereas the vaccination preparation administered to group 1 included about 30 .mu.g G-CSF. The vaccination preparations administered to groups 1 and 2 included aluminum hydroxide adsorbed G-CSF, but the vaccination preparation administered to group 3 included a MDP-Lip encapsulated G-CSF depot. Group 5 was the control group. The corresponding vaccination preparations were administered four times (4.times.) to the individuals in each vaccination group on days 35, 28, 21, and 14 prior to the challenge day. Group 5 received medium only on each one of the four vaccination days. All the administrations were subcutaneous, and the challenge comprised tumor-inducing B16 cells. These group characteristics are summarized in Table 19.

Detailed Description Text (203):
FIG. 22 shows that 60% of the individuals in groups 1 and 3 were alive on day 100 after challenge day, whereas only 40% in group had survived the same period of time. Neither the mice that had been administered medium nor the ones that had been vaccinated with

inactivated B16 cells only survived for more than 30 days after challenge day. These data indicate that G-CSF encapsulated in MDP-Lip may have greater adjuvant effects than G-CSF adsorbed to aluminum hydroxide because a greater dose of G-CSF had to be administered when it was adsorbed to aluminum hydroxide to achieve the same survival rate on day 100. Notably, even the best survival rate in this experiment is significantly lower than that achieved with the preferred preparation in this invention.

Detailed Description Text (229):
The influence of vaccination frequency on survival is analyzed in this B16 prophylactic study. Vaccination groups 1 and 2 comprised ten mice each, vaccination group 2 comprised mice and vaccination group 4 was the control group with six mice. About 10.sup.5 inactivated B16 cells were included in the vaccination preparations administered to mice in groups 1-3. The vaccination compositions administered to groups 1-2 included about 10 .mu.g IL-2 adsorbed to about 10 .mu.g of aluminum hydroxide. Group 4 was the control group. Vaccinations with the corresponding compositions were subcutaneously administered four times (4.times.) to individuals in vaccination group 2 on each one of days 35, 28, 21, and 14 prior to challenge day. Vaccination groups 1 and 3 received six vaccinations (6.times.); the two additional vaccinations were administered one on each one of days 49 and 42 prior to the challenge day. Mice in the control group received only medium on each of the vaccination days of groups 1 and 3. Vaccination group characteristics are summarized in Table 22.

Detailed Description Text (235):
In this RenCa therapeutic study a preparation comprising about 10 .mu.g IL-2 adsorbed to about 10 .mu.g of aluminum hydroxide was injected into tumors induced by injection of 10.sup.5 vital RenCa tumor cells (intratumoral treatment) every third day after the tumor became palpable. Mice were vaccinated between seven and ten times with about 100 .mu.l of the intratumoral treatment preparation every time. Saline solution with no further additions was administered to the animals in the control group. FIG. 28 shows that no mouse in the control group survived for more than 50 days after the tumor induction day. On day 100, a survival rate of 60% was observed in the group that received the intratumoral treatment preparation. The significance computed on day 100 is 0.0002, which indicates that the explanation that the more positive effects on the survival rate are due to the intratumoral 10 .mu.g IL-2 AL preparation should be accepted over the explanation that the increased survival rate is not due to this preparation with a significance of 0.02%. A comparison of the survival rates shown in FIG. 1 for treatment group 2 with the survival rate shown in FIG. 28 for the group that received intratumoral treatment preparation reveals that the effects of multiple intratumoral injections with a preparation that contained the cytokine IL-2 adsorbed to aluminum hydroxide were more positive than the single subcutaneous administration of the same preparation. However, the effects on survival rates of the single subcutaneous administration of the 10.sup.6 RenCa 10 .mu.g IL-2 AL preparation were more positive than the effects on survival rates of the multiple intratumoral administration of the intratumoral treatment preparation with 10 .mu.g IL-2 AL, as evinced by the comparison of the survival rates for group 5 in FIG. 1, group 1 in FIG. 2, group 5 in FIG. 8, and group 1 in FIG. 13 with the intratumoral treatment group 10 .mu.g IL-2 AL in FIG. 28. This is due to the fact that the tumors into which the intratumoral aluminum hydroxide adsorbed IL-2 was injected had a considerable size (they were "palpable") and contained more tumor cells than the tumor-inducing inoculum applied to the animals of , e.g., group 5 in FIG. 1.

Detailed Description Text (241):
The influence of spatial distribution of IL-2 containing vaccines on survival is analyzed in this B16 prophylactic study. Vaccination groups comprised six mice each. About 10.sup.5 inactivated B16 cells were included in the vaccination preparations administered to mice in groups 1-4. The vaccination compositions administered to group 1 included about 10 .mu.g IL-2, and the vaccination compositions administered to vaccination groups 2 and 3 included about 2.5 .mu.g IL-2 and about 10 .mu.g IL-2, respectively. Vaccination group 4 received inactivated B16 cells only, with no cytokine. Vaccination group 5 was the control group. Four vaccinations (4.times.) with the corresponding composition were subcutaneously administered to individuals in vaccination groups 1-4, one vaccination on each one of days 35, 28, 21, and 14 prior to challenge day. Individuals in vaccination group 1 received one vaccination (1.times.) dose at one location each one of the vaccination days. Individuals in vaccination groups 2, 3 and 4 received one vaccination dose at four locations every vaccination day.

Detailed Description Text (248):

The temporal in vivo release patterns of the following subcutaneous administrations have been investigated: 100 .mu.g IL-2 in free form; 100 .mu.g IL-2/100 .mu.g AL; 10 .mu.g IL-2/10,000 .mu.g CP, and 100 .mu.g IL-2 adsorbed to 1,000,000 tumor cells. The cytokine concentration was determined in peripheral blood during the follow-up period. It is to be understood that the cytokine concentrations determined at different times do not represent the actual release of cytokine at the injection site, but they instead provide readings for the amount of cytokine that is left over after the IL-2 molecules have left the inoculum, diffused through the tissue, penetrated the vascular system, and reached the peripheral blood stream. On route, IL-2 molecules will have bound to and interacted with molecules and cells in tissues and in blood and, consequently, the IL-2 concentration will be reduced considerably.

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